DESIGN, DEVELOPMENT AND EVALUATION OF FENOFIBRATE AND ROSUVASTATIN NANODROPLETS

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Abstract

Fibrate and statin combinational therapy is used to treat severe and refractory mixed hyperlipidemia. Hence lipid based SNEDDS were prepared using the concentration oil (2:1) and SCoS (1:1) which resulted in enhanced extent of absorption and relative bioavailability of 1.69 (fibrate) and 1.64 (statin). An approximately 40% of drug was available for systemic circulation via lymphatic route of absorption. The results showed a significant difference between the marketed and the SNEDDS

Keywords: Fibrates, statins, SNEDDS, hyperlipidemia, lymphatic absorption

Intoduction

Oral route is the most commonly preferred route for drug delivery of wide variety of drugs. BCS Class II drugs suffer from poor water solubility and high lipophilicity resulting in fluctuations in drug plasma level, influence by food, rapid metabolism leading to failure of conventional drug delivery systems (1). These drug exhibit variable bioavailability and need improvement in the dissolution rate by different methods like lipid formulations, amorphous formulations like solid dispersion systems etc., for improving bioavailability(2). The choice of formulation is often of critical importance in establishing a successful product for oral administration of a BCS class II drug. Even though they contain potential pharmacodynamic activity they fail to reach market (3). The solubility of the drug could be increased in three ways: changing the chemical structure in the lead optimization phase; prodrug approach and the formulation approach. Lipid based drug delivery systems (LBDDS) are gaining importance these days due to their ability to deliver drug via lymphatic route restraining the hepatic metabolism. LBDDS (4) are a diverse group of formulations which are classified into 4 types: Type I (oils without surfactants) , Type II(oils and water insoluble surfactants), Type III(oils, surfactants, co solvents), Type IV(water soluble surfactants and co-solvents). Of which Type III, popularly known as Self Nano Emulsifying Drug Delivery Systems (SNEDDS) are widely used because of its ease of formulation : simple self emulsification technique. SNEDDS (5) are defined as isotropic mixtures of natural or synthetic oils containing solid or liquid surfactants and one or more hydrophilic solvents. They form fine oil in water (o/w) emulsions on contact with GI fluids. The drug in SNEDDS remains in the solution form throughout its GI transit time whereby they circumvent the dissolution step. It involves digestion of the excipients and formation of different colloidal structures. The drug gets partitioned into these structures before it is absorbed. Thus they enhance the bioavailability of CLASS II drugs (6). SNEDDS improve the bioavailability by facilitating transcellular and paracellular absorption and inhibiting P-gp & CYP450 enzymes thereby decreasing intestinal efflux and drug biotransformation (7,8). The drug is absorbed through the lymphatics while chylomicrons are synthesized from the fatty components of the oil phase of the emulsion (9).

The main objective of the study is to formulate SNEDDS of the BCS Class II drugs, Fenofibrate and Rosuvastatin (10), as bioactives so that the drug can be delivered in the form of nanodroplets. Fenofibrate is an antilipidemic agent that reduces both cholesterol and triglycerides in the blood by activating peroxisome proliferator activated receptor a (PPARa). Rosuvastatin is an antilipidemic agent that competitively inhibits hydroxymethyl glutaryl-coenzyme A (HMG-CoA) reductase, which catalyses the conversion of HMG-CoA to mevalonic acid, the rate limiting step in cholesterol biosynthesis. Fenofibrate and Rosuvastatin combinational therapy is recomended for the treatment of severe or refractory mixed hyperlipidemia(11).

The present marketed formulations face bioavailability problems, affected by first pass metabolism and pose toxicity due to intake of high doses (10). Hence suggesting SNEDDS for formulating Fenofibrate and Rosuvastatin improves bioavailability, bypass first pass metabolism and thereby decreases the dose of the drug and hence the toxicity. The thus formulated nanodroplets are characterized and evaluated for *in-vitro*, *in-vivo* performance and lymphatic absorption. Orally administered highly lipophilic compounds reach systemic circulation via intestinal lymphatic system (12). This alternative pathway from GIT has been shown to be significant contributor for overall bioavailability of a number of highly lipophilic drugs. Lymphatic transport of the drug provides many advantages such as avoidance of hepatic first pass metabolism and improved plasma profile of the drug.

Materials and methods

Materials

Fenofibrate and Rosuvastatin drugs were obtained as a gift sample from Zim Laboratories, Nagpur; Capryol 90, Capmul MCM, Labrafac (LF), Isopropyl myristate (IPM), Lauroglycol (LG), Labrafac Lipophile WL 1349 (LL), Poloxamer 188, Cremophore EL, Cremophore RH 40, Gellusire 44/14 and Labrasol, Transcutol HP, Labrafil M 1944 CS were obtained as gift samples from Gattefosse Pvt. Ltd. (Mumbai, India). Neusilin was obtained from Fuji Chemical Industry Co. Ltd, Japan. Ethanol and Methanol (HPLC grade) were obtained from Merck chemicals India Pvt. Ltd., Mumbai. Avocadro oil was obtained from Swastik Eucalyptus Oil Co.

Methods

A Solubility studies (13)

Solubility of Fenofibrate and Rosuvastatin was determined in various oils such as Capryol 90, Capmul MCM, LF, IPM, LG, LL, by shake flask method (14). An excess amount of drug was taken in 10 ml of the oil in vials, and mixed using vortex mixer. The vials were then kept at 25 ± 1^0C in an isothermal shaker for 72hrs to reach equilibrium. The equilibrated samples were then centrifuged at 3000rpm for 15min. The supernatant was filtered through a 0.45µm membrane filter. The concentration of the drug (Table 1) was determined using UV Spectroscopy at 286.5nm and 243.5nm respectively.

(Compatibility studies were performed using FTIR 8400 S, Shimadzu. The IR spectrum of the physical mixture was compared with those of pure drug, lipid and surfactants and peak matching was done to detect any appearance or disappearance of peaks).

Methodology (15)

SNEDDS were formulated by spontaneous emulsification technique using slow aqueous titration

Construction of pseudo ternary phase diagrams (16, 17)

Pseudo ternary phase diagrams were constructed to examine the formation of oil in water nanoemulsion with 4 components oil, surfactant, co surfactant, and aqueous phase. The 4-component system consisted of (i) Capryol 90 and Capmul MCM 2:1 (Selected from solubility studies) (ii) surfactant Cremophore RH 40 with HLB value 12.5 (iii) a Co surfactant (Ethanol) and (iv)distilled water (aqueous phase). Surfactant and co surfactant mixture (SCoS) in each group were mixed in different volume ratios (1:0, 1:1, 1:2, 1:3, 2:1, 3:1, 4:1**)**. Seventeen combinations of oil and SCoS, 1:9, 1:8, 1:7, 1:6, 1:5, 1:4, 1:3, 1:2, 1:1, 5:1, 4:1, 3:1, 2:1 were made so that maximum ratios were covered for the study to depict the boundaries of phases precisely formed in the phase diagrams Fig (1-2). Slow titration with aqueous phase (18**)** was done to each volume ratio of oil and SCoS and visual observation was carried out for transparency and flowability of the nanoemulsion.

Different concentrations of oil from NE region of phase diagrams were selected, and the drug is incorporated. For each % of oil selected, the formula that used the minimum concentration of SCoS for its NE formulation was selected.

Characterisation of nanoemulsion (19,20)

The formulations were subjected to different thermodynamic stability studies (Table 2) such as centrifugation, heating cooling cycle (21) and freeze thaw cycle, to avoid the selection of metastable formulations. Thermodynamic stability of nanoemulsion differentiates them from those systems which are kinetically stable and eventually phase separate. The dispersibility of the nanoemulsion was assessed using a standard USP XXII dissolution apparatus II. The *in vitro* performance of the formulations was visually assessed by phase clarity, self emulsification time, and rate of emulsification. Rapidly forming (within 1min) nanoemulsion, having a clear or bluish appearance, rapidly forming, slightly less clear emulsion, having a bluish white appearance were selected for further studies (22) (Table 3**).** The selected formulations were prepared by dissolving 67 mg (single dose) of Fenofibrate and 5mg of Rosuvastatin in oil (10%, 15%, 20%, 25% etc.). Respective SCoS ratio was added to the oil, and mixed using vortex mixer.

The formulated SNEDDS were evaluated for the following parameters *in vitro* (table 3).

SLNO	PARAMETER	METHOD
1	Globule size & polydispersity index	Zetasizer 3000 (Malvern Instruments Worcestershire, UK) (fig 6)
2	External Morphological Study	Scanning Electron Microscopy(fig 7)
3	Viscosity determination	Brookfield DV-II ultra+ viscometer
4	Electroconductivity study	Electroconductometer (Conductivity meter 305, Systronic).
5	Refractive index and % transmittance	Abbe refractometer & UV spectrophotometer.
6	*In vivo* performance	50,100, 1000 times dilution with various dissolution media viz: Water and Simulated gastric fluid (pH 1.2).
7	Drug content	UV Visible spectrophotometry.

From the stock solution of Fenofibrate and Rosuvastatin, dilutions were prepared and the calibration curve in acetonitrile (ACN) constructed between concentration and peak area.

Chromatographic conditions for simultaneous estimation of Fenofibrate and Rosuvastatin

The following is the optimized chromatographic conditions were selected for the simultaneous estimation of Fenofibrate and Rosuvastatin (23). Shimadzu gradient HPLC system was used with following configurations: stainless steel column (Inertsil ODS, 250 x 4.6mm, 5µ column), which was maintained at 25^{o}C. The dual analytical were set, 248 nm for Rosuvastatin and 286 nm for Fenofibrate and samples of 5µl were injected to HPLC system. The mobile phase was a mixture of water (pH 2.5 adjusted with ortho-phosphoric acid) and

acetonitrile in ratio of 30:70 (v/v) at a flow rate of 1.0ml/min. The mobile phase was filtered through 0.45µm filter (Sartorius, Germany) and degassed for 10 minutes by sonication.

***In vitro* drug release in simulated gastric fluid pH 1.2**

The quantitative *in vitro* release test (15) was performed in 250 ml pH 1.2 simulated gastric fluid using USP dissolution apparatus Type I at 50 rpm at 37 ± 0.5^0c. The Optimized SNEDDS formulation containing single dose of Fenofibrate and Rosuvastatin were filled in Size 1cs of Hard gelatin capsule (24, 25) (CONISNAP). Samples were withdrawn at regular time intervals (0, 0.5, 1, 1.5, 2, hrs) and an aliquot amount of dissolution media was replaced. The release of drug from SNEDDS formulation was compared with the conventional tablet formulation and the samples were analyzed for the drug content (table 4)**.**

***In vivo* bioavailability studies (26)**

The experiments were carried out after getting the approval of the CPCSEA and IAEC, JSS College of Pharmacy, Ooty.Proposal no: JSSCP/IAEC/M.PHARM/PH.CEUTICS/01/2012-13 and their guidelines were followed throughout the experiment.

Estimation of drug in rabbit plasma

Healthy overnight fasted Male Sprague-Dawley rats weighing about 350-370gm were used for both *in-vitro* and *in-vivo* the experiments. The animals were given water *ad.libitum* during fasting and throughout the experiment. Zero hour fasting blood samples were withdrawn early in the morning. The animals were then divided into 2 groups each containing two animals. The dose for the rabbits was selected based on the surface area ratio of rabbit and man; and the dose was administered with an oral cannula. Group 1 received drug conventional marketed tablet formulation and group 2 received SNEDDS. Immediately after administration the animals were given 5ml of water. The animals were anaesthetized and the carotid artery is cannulated (27).

Blood samples (0.5ml) were withdrawn from the carotid artery at 0,0.25,0.5,0.75,1,2,4,6,8,12 hours with a sterile syringe. The blood samples were collected in a ria vial containing anti-coagulant (0.4 ml of 2.5% sodium citrate), centrifuged at 2500rpm for 4 min and the plasma samples were separated and stored at -20^0C. The plasma samples were deprotonated and extraction of drug was done by solid phase extraction (SPE) using ACN-water mixture and analyzed. Estimation of plasma samples by HPLC was carried out using optimized chromatographic conditions mentioned earlier (table 5).

Pharmacokinetic data analysis

Predicting the release behavior of the drug *in vivo* can be done by pharmacokinetic treatment of data. Pharmacokinetic parameters (table 6) after oral administration of Olanzapine in rabbits were calculated by Wagner-nelson method. The pharmacokinetic data was analyzed by one-way analysis of variance (ANOVA) using Tukey-Kramer method.

***In-vivo* lymphatic absorption studies (28)**

All the animals were suitably anesthetized, and cannulation of mesenteric lymph duct for collection of intestinal lymph and duodenum for the administration of rehydration solution was done as per the procedure (27). To study the lymphatic absorption, the lymph that drains from the intestinal lymphatic duct was collected at predetermined time intervals for 12 hrs, analyzed for drug concentration and multiplied with the volume of the lymph collected(fig 4).

Results and discussion

Solubility studies of drug in different oils

Solubility is an important criterion in formulation of SNEDDS, as the drug remains in the liquid form solubulized in the oil phase. Hence the oil phase in which the drug shows maximum solubility is to be selected for the purpose. From table 1 it was evident that Capryol 90 shows maximum solubility of Fenofibrate i.e 180 mg/mL and Capmul MCM shows maximum solubility of Rosuvastatin i.e 30 mg/mL. Hence the combination of Capryol 90 and Capmul MCM in the ratio of 2:1 (which was calculated based upon the dose of the drugs) was selected for the formulation of SNEDDS.

Table 1: Solubility studies

OIL	FENOFIBRATE SOLUBILITY(mg/mL)	ROSUVASTATIN SOLUBILITY(mg/mL)
CAPRYOL 90	270	30
CAPMUL MCM	110	60
LF	96	42
IPM	72	21
LG	84	24
LL	126	44

Pseudo ternary phase diagram study

When surfactant alone (SCoS 1:0) was used only a small area of nanoemulsion is formed with oil solubulized upto 18% and SCoS 65%, while surfactant and co surfactant were taken in equal ratio (SCoS 1:1) there was a slight increase in nanoemulsion region but the oil solubulization increased upto 23% with 59% of SCoS. This could be due to the addition of co-surfactant which leads to greater penetration of oil phase into the surfactant thereby decreasing the interfacial tension.

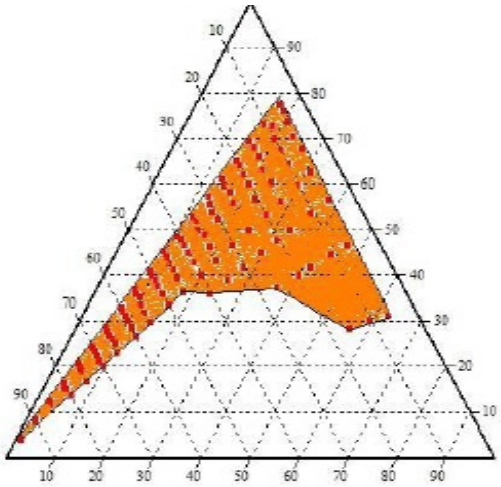


Fig 1: SCoS 1:1

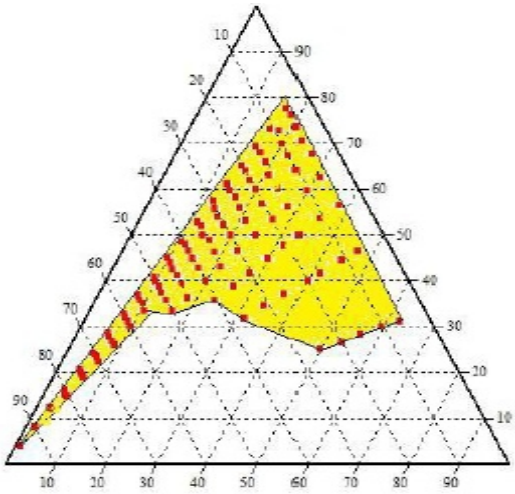


Fig 2: SCoS 1:2

When the concentration of co-surfactant is doubled (SCoS 1:2) nanoemulsion area increased considerably with 30% oil solubulized with 55% SCoS. There was no difference in nanoemulsion region and the oil solubulization remained same for (SCoS 1:3). But on further increase of co-surfactant (SCoS 1:4) the oil solubulization decreased to 28%.

When Surfactant concentration (SCoS 2:1) was doubled nanoemulsion area was large and the maximum oil solubulization was up to 32% with only 52% of SCoS. A further increase in surfactant concentration i.e., SCoS 3:1 resulted in decrease of oil solubulization of only up to 25%. For SCoS 4:1 even a high concentration of SCoS 65% resulted in less nanoemulsion area with only 19% of oil solubulization.

Thermodynamic stability studies and dispersibility tests

The selected formulations from the pseudo ternary phase diagrams were subjected to different thermodynamic stability studies and the formulations which survived thermodynamic stability studies were taken for dispersibility test. (Table no: 2). Thermodynamic stability studies differentiate those nanoemulsion formulations from those of kinetically stable and undergo phase separation. This implies that the formulations contain adequate amounts of SCoS concentration required for nanoemulsion formulation, which decreases the energy required for nanoemulsion formation. This decreased energy contributes to the stability of nanoemulsion. The nanoemulsion formulation on entering the GI tract undergoes infinite dilution leading to phase separation of the formulation due to poor aqueous solubility of the drug.Formulations which passed the dispersibility studies were certain to remain as nanoemulsion upon dispersion in the aqueous environment of the GIT. For oral nanoemulsion the process of dilution by the GI fluids will result in the gradual desorption of the surfactant located at the globule interface. The process is thermodynamically driven by the requirement of the surfactant to maintain an aqueous phase concentration equivalent to its critical micelle concentration.

SCoS	Oil %	SCoS %	Aq %	Centrifugation	H/C cycle	Freeze Thaw	Dispersion
1:0	10	30	60	F	P	P	-
	15	56	29	P	F	F	-
1:1	10	18	72	P	P	P	-
	15	25	60	P	P	P	***
	20	36	54	P	P	P	-
	25	45	30	P	P	P	***
1:2	10	27	63	P	F	P	-
	15	33	52	P	P	P	***
	20	37	43	P	P	F	-
	25	44	31	P	P	P	-
	30	54	16	P	P	P	***
1:3	10	18	72	P	F	F	-
	15	19	66	P	P	P	-
	20	30	50	P	P	P	-
	25	36	39	P	P	F	-
	30	51	19	F	P	P	-
1:4	10	18	72	P	P	P	-
	15	21	64	P	P	P	-
	20	35	45	P	P	P	-
2:1	10	22	68	F	P	F	-
	15	25	60	P	P	P	-
	20	33	47	P	F	P	-
	25	40	35	F	P	P	-
	30	45	15	P	P	P	-
3:1	10	18	72	P	P	P	-
	15	25	60	F	P	P	-
	20	32	48	P	P	F	-
4:1	10	20	70	F	P	P	-
	15	25	60	P	F	P	-

Table no: 2 Thermodynamic stability studies and dispersibility test.
*** Formulation which passed the dispersibility test; P- pass; F- fail.

Formulations which passed thermodynamic stability tests and dispersibility test were subjected to globule size analysis, refractive index determination, viscosity determination and *in vitro* release studies.

Characterization studies

Optimized formulations selected from phase diagram at a difference of 5% w/w of oil having least SCoS concentration that passed dispersibility test were selected, named as formulation A,B,C, and D. They were subjected to *in vitro* characterization studies (Table 4).

The globule size increases with increase in concentration of oil in formulation and decreases with increase in the concentration of SCoS. The mean globule size of the formulation A containing 25% oil was 90 nm while as formulation, B containing 30% oil was 254.3nm and formulation D with 15% oil was 40 nm. Since the globule size of the droplets was much smaller than that of the blood capillaries (400nm), there are minimal chances of capillary blockage during transport of the droplets. Thus higher circulation time of the droplets after *in vivo* application is also favored. The polydispersity index (PDI) of formulation A and C was 0.287 and 0.398 suggesting uniformity in the globule size of the formulation but in the case of formulation B and D the PDI was 0.723 and 0.910 so these formulations were dropped from further studies.

Zeta potential of a formulation relates to its colloidal stability. It indicates the degree of repulsion between adjacent and similarly charged particles in the dispersion. For molecules that are small enough a high zeta potential confers stability and resists aggregation. The zeta potential of the formulations A and C were -19.0 and -22, indicates that the formulations are stable.

The viscosity of the optimized formulations was determined. It was observed that viscosity of all the formulations is less than 31 cp. Formulation A and C has the minimum viscosity 22.3cp and 19.2cP, while B has highest viscosity of 30.1cp perhaps because of its higher oil content. Lower viscosity is an ideal characteristic of the o/w nanoemulsion.

Conductivity of the optimized formulations was found in range of 451-522.3 μS/cm**.** From the viscosity and the electro-conductivity study it is concluded that the system is of o/w type.

The refractive index of the developed system was similar to the refractive index of the water (1.333). In addition, the developed system showed percent transmittance > 97%. The observed transparency of the system is due to the fact that the maximum size of the droplets of the dispersed phase is not larger than 1/4 th of the wavelength of visible light**.** Thus, NE scatters little light and was therefore transparent or translucent.

Fenofibrate and Rosuvastatin from the SNEDDS were extracted by dissolving in ACN, analyzed spectrophotometrically against solvent blank. Drug content of the optimized formulations were found to be 70mg, 5mg; 120mg, 8.5mg; 70mg, 5mg; 140mg,10mg for formulations A, B, C, D respectively.

Table 3: Characterization of Nanoemulsion

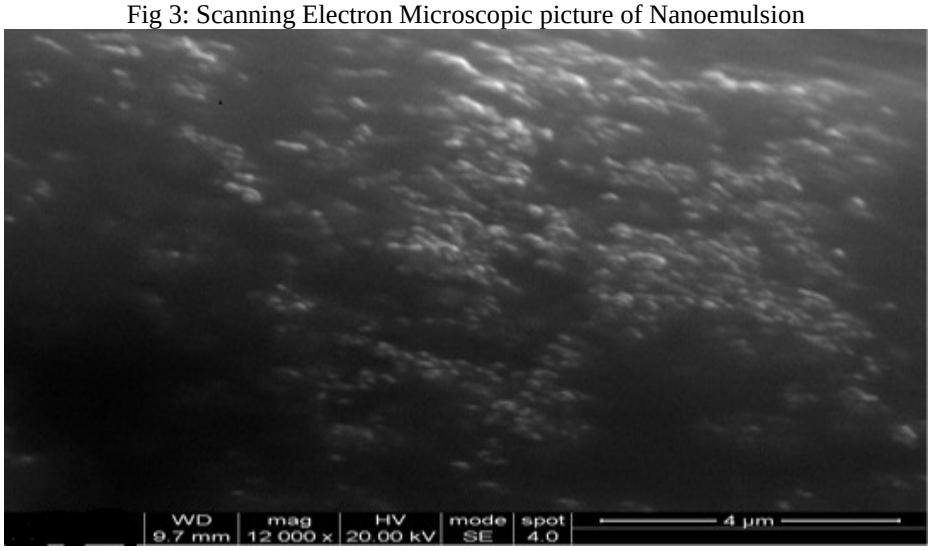
Formulation	A	B	C	D
SCoS(ml)	1:1	1:1	1:2	1:2
Oil %	15	25	15	30
S%	12.5	22.5	11	18
Cos%	12.5	22.5	22	36
Aq%	60	30	52	16
Globule size(nm)	90	254.3	212	40
PDI	0.287	0.723	0.398	0.910
Zeta Potential(mV)	-19.0	-17	-22	-5
Viscosity(cP)	22.3	30.1	19.2	-
Conductivity(μS/cm)	451.3	-	522.3	-
Refractive Index	1.49	-	1.27	-
%Transmittance	99.43	-	98.9	-
Drug content(mg/mL) Fenofibrate	70	120	70	140
Rosuvastatin	5	8.5	5	10

From the above analysis the formulation A was selected for drug incorporation and *in vitro* and *in vivo* studies.

Scanning electron microscopy (SEM)

The scanning electron microscopic study reveals the external morphology of the nanoparticles and from fig 3 it was evident that maximum nanoparticles were nearly spherical in shape.

Fig 3: Scanning Electron Microscopic picture of Nanoemulsion



***In vitro* drug release**

In vitro dissolution studies were performed in pH 1.2 simulated gastric media. Comparative dissolution studies were performed to investigate the drug dissolution from SNEDDS and marketed tablet formulation containing same quantity of drug. *In vitro* dissolution studies showed that Fenofibrate is rapidly released ≥98 % and ≥96% of Rosuvastatin from the optimized SNEDDS. Initially during the first 15 min, the SNEDDS had shown a release of ≥38% of Fenofibrate and ≥33% of Rosuvastatin, but by the end of 30min, there was a drastic change in the release behavior which was upto ≥64% of Fenofibrate and ≥56% of Rosuvastatin. Out of this ≥80% of the drug released during first hour of the study. In contrast, the marketed formulation has shown a release of ≥ 60%. This is because of the small globule size, and eventually higher surface area in case of SNEDDS, which permits faster rate of drug release. Being droplets and the drug in solution form, instant absorption should have been taken place. Since the carrier is lipid, it undergoes lipolysis in the presence of bile salts and pancreatic lipases etc. Therefore the surfactant layer around the droplets gets released and is converted to micelles, reverse micelles etc. Sometimes this may lead to precipitation of drug since the drug gets detached from the droplets. This is the case where the surfactants are directly involved in the solubility of the drug. Infact the total amount of drug release is delayed due to this lipolysis process.

Table no: 4 *In vitro* dissolution data in pH 1.2 simulated gastric fluids.

Time (min)	% Cumulative release			
	SNEDDS		Marketed formulation	
	Fenofibrate	Rosuvastatin	Fenofibrate	Rosuvastatin
15	38.21	33.17	32.33	29.9 1
30	64.61	56.28	49.49	45.18
45	75.37	63.41	56.32	52.42
60	80.37	81.37	60.36	59.36
90	95.37	93.23	64.00	62.10
120	98.95	96.64	68.30	64.20

In vivo bioavailability studies

In vivo bioavailability studies were performed to quantify SNEDDS formulation after oral administration and to compare the bioavailability of SNEDDS with that marketed formulation. From the table 5 it can be inferred that the SNEDDS formulation showed maximum plasma concentration of 80% by the end of first hour while the pure drug suspension and marketed formulations showed only 54 and 57% release by that time respectively. The pure drug suspension and marketed formulations took 2hours to reach maximum plasma concentration of 56 and 61% respectively. From the above analysis it is evident that the SNEDDS formulation showed improved rate of drug release compared to the conventional marketed formulations. The SNEDDS showed maximum drug concentration in half of the time taken by the pure drug suspension and the marketed formulation.

Table no: 5 Comparative *in vivo* bioavailability studies

Time (hrs)	Marketed Formulation (ng/h/ml)		SNEDDS (%)	
	Fenofibrate	Rosuvastatin	Fenofibrate	Rosuvastatin
0.25	21.44 ± 1.25	1.55±0.98	32.16±1.80**	2.05±0.92**
0.5	24.79 ± 1.71	1.79±0.52	48.64± 2.41***	3.23±0.16**
0.75	28.81 ± 1.90	2.13±0.85	53.6±2.76***	3.7±0.02***
1	32.83 ±1.92	2.31±0.64	56.54±2.14**	4.16±0.56***
2	35.175 ± 1.13	2.38±0.09	54.002±2.18**	3.96±0.80**
4	32.75 ± 1.74	2.21±0.62	48.48±1.79**	3.51±0.62**
6	26.66 ±1.79	1.85±0.33	42.96 ±1.74**	2.91±0.32**
8	24.12 ±1.51	1.70±0.02	37.25±1.58**	2.03±0.43**
12	21.44 ±1.64	1.45±0.56	22.38±1.19**	1.51±0.72**

Values are expressed as mean ±S.D; n=3

*P<0.05; **P< 0.01;***P<0.001 when compared with marketed formulation.

One way ANOVA followed by TUKEY-KRAMERS multiple comparison tests.

Pharmacokinetic treatment

Pharmacokinetic parameters were calculated from the *in vivo* release of Fenofibrate and Rosuvastatin in Sprague-Dawley rats for SNEDDS, and marketed tablet using Wagner-Nelson method (table 6).

Since the rate limiting step in the absorption of Fenofibrate and Rosuvastatin was dissolution from the formulation and the results from the study reveals that the dispersion of the drug (since the drug is completely dissolved in the oil phase) into the aqueous gastrointestinal environment is the rate limiting step in case of SNEDDS and plays a major role for absorption. It can be explained that, following oral administration, SNEDDS disperse spontaneously to form a nanoemulsion in the GI fluid where the active components are present in a solubulized form, and the small droplet size provides a large surface area for drug absorption. Such an ultra fine dispersion of the oil will afford rapid and extensive absorption. In addition high concentration of surfactant in SNEDDS may increase permeability of the oil across the cell membrane, and lymphatic transport through the transcellular pathway. As the lipolysis proceeds amount of drug available for absorption is also more. This could be the reason for the formulation to take around 1hr to achieve $T_{max.}$ Whereas the conventional marketed formulation did not result in the same manner. Fact may be that since it takes more time for disintegration and to go into the solution. Hence it took more than 2hrs to achieve T_{max}, and moreover the extent of absorption which is evident from AUC_{0-t} and $AUC_{0-\infty}$, the SNEDDS achieved maximum which is statistically significant.

Table 6: Pharmacokinetic parameters

Pharmacokinetic parameters	**Marketed formulation**		**SNEDDS**	
	Fenofibrate	Rosuvastatin	Fenofibrate	Rosuvastatin
T max(h)	2	2	1	1
K_e	0.283±0.0153	0.245±0.0124	0.7343±0.0162**	0.944±0.0132**
C_{max}(ng/ml)	35.175±1.13	2.38±1.64	56.548±2.14**	4.16±2.56**
AUC_{0-t}(ng h/ml)	326.1013±22.56	22.50125±2.48	489.305±98.9*	32.735±8.28*
$AUC_{0-\infty(}$ng.h/ml)	401.86±21.6	28.4196±23.8	519.78±59.35*	34.335±55.45*
F_r	-----	-----	1.69	1.64

Values are expressed as mean ±S.D; n=3

*P<0.05; **P< 0.01;***P<0.001 when compared with marketed formulation.

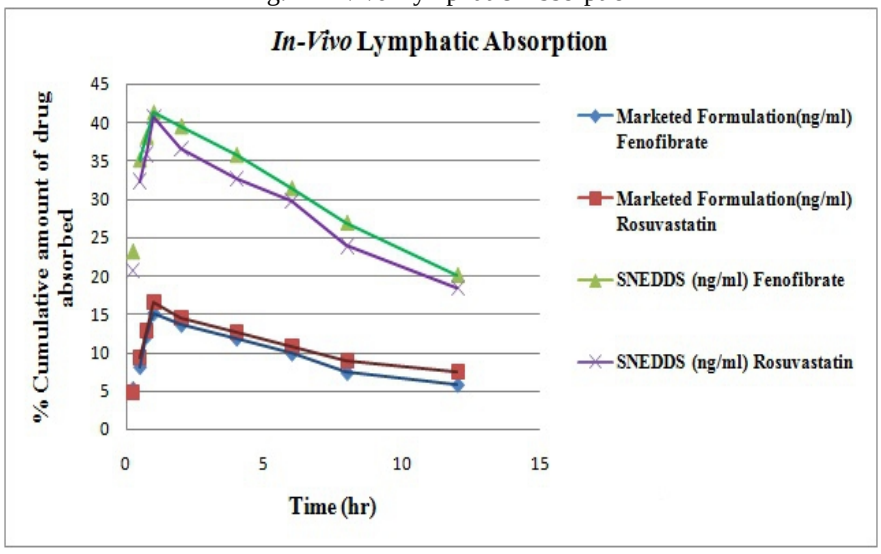
One way ANOVA followed by TUKEY-KRAMERS multiple comparison tests.

The relative bioavailability of the SNEDDS was 1.69 and 1.64 for Fenofibrate and Rosuvastatin which is 0.69 and 0.64 folds increase when compared to marketed formulation. From the above discussion it was evident that SNEDDS showed improved bioavailability and drug disposition compared to the marketed formulation. This results shows the lymphatic absorption plays a major role in enhanced bioavailability of lipid formulations i.e SNEDDS.

In vivo lymphatic absorption studies

In vivo lymphatic absorption studies in Sprague-Dawley rats were performed and the intestinal lymphatic fluid was collected for a period of 12hrs. The amount of drug that has been absorbed through lymphatic system was found to reach a maximum of 41% by 2 hr after administration of drug while the . From then the rate at which drug absorbed via lymphatic route showed a decline. This is due to the lipid nature of the droplets, micelles and reverse micelles formed during lipolysis. It is also evident that the result of lipolysis did not account for precipitation. Hence maximum amount of drug was available for absorption. Moreover the lipid carriers and the surfactants might have involved in overcoming the P-gp efflux, thereby increasing the drug available for absorption which is evident from the lymphatic drug absorption study.

Fig: 4 *In-vivo* Lymphatic Absorption



Conclusion

By formulating Fenofibrate and Rosuvastatin as the SNEDDS (with appropriate selection of oil (2:1) and SCoS(1:1)) in combination, the problems faced by the conventional formulations could be overcome. Since being a lipid formulation enhanced bioavailability by lymphatic system mediated drug absorption is possible.

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